



THE UNIVERSITY *of* EDINBURGH

## Edinburgh Research Explorer

### New developments in osteoarthritis and cartilage biology

**Citation for published version:**

Poulet, B & Staines, K 2016, 'New developments in osteoarthritis and cartilage biology', *Current Opinion in Pharmacology*, vol. 28, pp. 8-13. <https://doi.org/10.1016/j.coph.2016.02.009>

**Digital Object Identifier (DOI):**

[10.1016/j.coph.2016.02.009](https://doi.org/10.1016/j.coph.2016.02.009)

**Link:**

[Link to publication record in Edinburgh Research Explorer](#)

**Document Version:**

Publisher's PDF, also known as Version of record

**Published In:**

Current Opinion in Pharmacology

**General rights**

Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

**Take down policy**

The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact [openaccess@ed.ac.uk](mailto:openaccess@ed.ac.uk) providing details, and we will remove access to the work immediately and investigate your claim.





# New developments in osteoarthritis and cartilage biology

Blandine Poulet<sup>1</sup> and Katherine A Staines<sup>2</sup>

Osteoarthritis (OA) is a degenerative joint disease and the most common form of arthritis. Characterised by articular cartilage loss, subchondral bone thickening and osteophyte formation, the OA joint afflicts much pain and disability. Whilst OA has been associated with many contributing factors, its underpinning molecular mechanisms are, nevertheless, not fully understood. Clinical management of OA is largely palliative and there is an ever growing need for an effective disease modifying treatment. This review discusses some of the recent progress in OA therapies in the different joint tissues affected by OA pathology.

## Addresses

<sup>1</sup> Institute of Ageing and Chronic Disease, University of Liverpool, L7 8TX Liverpool, UK

<sup>2</sup> Roslin Institute and R(D)SVS, The University of Edinburgh, Easter Bush, Midlothian EH25 9RG, UK

Corresponding author: Poulet, Blandine ([b.poulet@liverpool.ac.uk](mailto:b.poulet@liverpool.ac.uk))

Current Opinion in Pharmacology 2016, 28:8–13

This review comes from a themed issue on **Musculoskeletal**

Edited by **Isabel R Orriss** and **Vicky E MacRae**

<http://dx.doi.org/10.1016/j.coph.2016.02.009>

1471-4892/Published by Elsevier Ltd.

## Introduction

With our ever ageing population comes a significant increase in the incidence of musculoskeletal disease and an acute need for effective therapeutic interventions. Osteoarthritis (OA) is a degenerative joint disease and the most common form of arthritis with 33% of people aged 45 years and over seeking treatment for OA in the UK. It is therefore a massive world-wide healthcare and financial burden. Characterised by articular cartilage (AC) loss, subchondral bone thickening and osteophyte formation, the OA joint afflicts much pain and disability [1].

Whilst OA has been associated with many contributing factors including ageing, obesity, trauma, genetics, amongst others, its underpinning molecular mechanisms are, nevertheless, not fully understood; indeed it is even still a matter of debate as to which is the precipitating pathology. Now regarded as a disease of the whole joint,

clinical management of OA is largely palliative with the use of opioids, non-steroidal anti-inflammatory drugs (NSAIDs) and steroid injections. In some individuals, only prostheses can offer long-term aid.

Clinical trials to date have included glucosamine sulfate, chondroitin sulfate, sodium hyaluronan, doxycycline, and matrix metalloproteinases (MMP) inhibitors; all of which have varying levels of efficacy and none of which have successfully and reproducibly prevented OA disease development or progression. As such, there is an ever growing need for an effective disease modifying treatment. Whilst our understanding of the aetiopathology of OA is dramatically advancing, few advances have been made in the pharmacological intervention of disease progression. This review discusses some of the most recent progress in OA therapies in the different joint tissues affected by OA pathology that were not already discussed by the recent review published in this journal in 2015 [2<sup>••</sup>].

## Targeting AC maintenance

AC degradation is one of the main hallmarks of OA development and to date, research has largely sought to identify those factors that target the AC to produce its disease-defining deterioration. However, the lack of blood vessels and the high ratio of extracellular matrix to cell area make this tissue difficult to target for repair; indeed pharmaceutical interventions are usually reliant on blood circulation of compounds through the body and to the cells through the matrix. An elegant and comprehensive review in this journal has recently summarised the most recent advances in finding new targets for reducing AC degradation, including cartilage degradation, autophagy, circadian clock, mechanical, inflammatory, oxidative stress, innate immunity, chondrocyte hypertrophy, pain [2<sup>••</sup>]. In addition to those mentioned by Goldring and Berenbaum [2<sup>••</sup>], further targets have recently been examined and are discussed herein.

Oxidative stress is emerging as a main contributor to OA severity. Recently, pathways centred around Heme Oxygenase 1 (HO-1), a major anti-oxidant, have been shown to play a major role in the oxidative stress response in chondrocytes. Indeed, Bach-1 (BTB and CNC homology) is a negative regulator of HO-1, and its deletion in mouse chondrocytes *in vivo* was shown to protect from OA development, via the promotion of HO-1 and autophagy [3<sup>•</sup>]. In addition, Nrf2 (nuclear factor (erythroid-derived 2)-like 2) is a promotor of HO-1 expression, and its

deficiency in mice lead to more severe OA development [4]. This effect was reduced with a histone deacetylase inhibitor (trichostatin A) following Nrf2 activation and HO-1 expression. Sulfuraphane was used a potent activator of Nrf2, inducing HO-1 expression, and decreased cartilage degradation in a murine post-traumatic model of OA [5]. These studies support HO-1 as a potent new target for cartilage degradation in OA.

TGF $\alpha$  (Transforming Growth Factor- $\alpha$ ) is a member of the epidermal growth factor (EGF) family and has been shown to be increased by 4 fold in the AC of OA rats [6]. It was subsequently found that TGF $\alpha$  can reduce chondrocyte anabolism while increasing catabolic processes and thus was proposed to be a potential target for therapy for AC degradation in OA development [7<sup>••</sup>]. Recently, Appleton *et al.* (2015) used pharmacological tools, namely AG1478, to inhibit TGF $\alpha$  *in vivo* in a rat OA model [8]. AG1478 was able to reduce cartilage degradation and OA severity, as well as increasing the levels of CPII (C-propeptide of collagen type II) in the serum while decreasing C2C (collagen type II breakdown product) levels, markers of cartilage anabolism and catabolism respectively. This study is one of the first to show pharmacologic efficacy in blocking post-traumatic OA development *in vivo*.

AC matrix degradation products have been shown to promote further joint degeneration. Indeed, fragments of collagen type II, aggrecan and fibronectin can induce further degradation through upregulation of MMP activity [9–11]. However, a complex interaction between specific fragments and concentration and mechanical stress may result in anabolic responses as well [12]. The signalling involved in these responses seems to be similar between cell types: indeed, Lees *et al.* [13] recently described the effect of an aggrecan 32-mer fragment derived from ADAMTS (A Disintegrin And Metalloproteinase with Thrombospondin Motifs) and MMP cleavage of aggrecan on chondrocytes, synovial fibroblasts and macrophages [13]. Treatment with this aggrecan 32-mer fragment resulted in pro-catabolic, anti-anabolic and pro-inflammatory activities, which were all abrogated in the absence of MyD88 (myeloid differentiation primary response gene 88), and was achieved via Toll-Like Receptor 2-dependent activation of the signalling pathway NF $\kappa$ B (nuclear factor kappa-light-chain-enhancer of activated B cells). This same pathway was also shown to be responsible for Fibronectin fragment catabolic responses from chondrocytes, suggesting potential targets for slowing the progressive degeneration of the AC in OA [14].

Although very informative, these studies have been performed in animal models, and thus further trials into human tissues and patients will be required to truly understand the potential of these targets for therapy.

## Targeting the subchondral bone

Subchondral bone pathologies in OA joints, although often considered secondary, are one of the earliest detectable changes and are now considered to be a potential trigger for subsequent OA progression [15,16]. These pathologies include sclerosis leading to joint space narrowing, with associated hypomineralisation and inferior bone quality due to abnormal local bone remodelling. It is therefore unsurprising that pharmacological interventions for the subchondral bone in OA have focussed on targeting regulation of osteoclast/osteoblast activity and as such, the bone remodelling process.

### Inhibition of osteoclast activity

Bisphosphonates are potent inhibitors of osteoclast activity, and are widely used in clinical practice to prevent the bone loss associated with conditions such as Paget's disease, metastatic bone disease and osteoporosis [17]. The use of bisphosphonates as means of OA therapies has been well investigated over the past decade with varying efficacy, and as such, more recent pharmacological studies have focussed upon the timing at which treatment with anti-resorptive agents should be used for disease modification [18<sup>••</sup>]. Pamidronate disodium (PAM) is a bisphosphate which completely prevents OA pathology in rabbits undergoing early anterior cruciate ligament transection (ACLT)-induced OA when administered short-term post ACLT. Similarly, long-term PAM administration reverses OA pathology in this model. This is therefore suggestive that PAM can significantly inhibit and even reverse early OA subchondral bone pathology, thought to be through OPG:RANKL (Osteoprotegerin — Receptor Activator of NF $\kappa$ B Ligand) mediated regulation of osteoclastogenesis [19]. Similarly, the preemptive use of another bisphosphonate, Alendronate, in a rat model for severe OA prevents OA bone pathologies including reduced subchondral bone loss and reduced osteophyte formation when compared to non-alendronate treated rats. Alendronate treatment also reduced AC degeneration, suggesting that osteoclastic activity drives AC degeneration [20]. Similar chondroprotective effects of the bisphosphonates clodronate and zoledronic acid have been reported on bovine chondrocyte cultures and ACLT in rabbits, respectively [21,22].

Whilst animal studies are informative, clinical trials are required to investigate the true therapeutic value of bisphosphonates in human OA. In the past two years there have been four clinical trials detailing the effects of bisphosphonates on human OA, with varying results. Nishii *et al.* found that 2 years alendronate treatment in patients with symptomatic hip OA revealed clinical efficacy for decreasing pain. Despite this, no differences were observed in OA disease pathology, as determined by radiographic measurements of Kellgren-Lawrence score, joint space narrowing and centre-edge angle [23]. Similar advantages for bisphosphonate use in improving pain

symptoms were reported in patients undergoing 4 weeks clodronate treatment for symptomatic knee OA [24]. A more recent randomised controlled trial investigating patients for 7 years following total knee replacement did however find that a year post-operative alendronate treatment significantly changed the mean bone mineral density of the femoral metaphysis up to four years following surgery, suggestive that alendronate treatment may be of benefit to patients following total knee replacement surgery [25<sup>••</sup>]. Similarly, another study examining the progression of OA found a trend to less joint space narrowing over time in bisphosphonate users in comparison to non-users [18<sup>••</sup>].

Together this suggests that bisphosphonates may have beneficial symptomatic and structural benefits for patients with OA, however many larger clinical studies are required to evaluate their true potential.

### Modifications of osteoblasts activities

As well as targeting osteoclasts in bone remodelling, studies have sought to identify whether the osteoblast is key to OA therapies. In the natural occurring OA guinea pig model, subcutaneous injections of Parathyroid Hormone (PTH) at a dosage of 15 µg/kg/day for five days/week for 3 and 6 months has advantageous effects on OA progression through decreasing the deterioration of the subchondral bone trabeculae and ultimately preventing AC degradation [26]. Similarly, in a rabbit model in which cylindrical osteochondral defects were created in the femoral trochlea, PTH treatment stimulated both subchondral bone and AC repair [27<sup>•</sup>]. Consistent with this, 2 year treatment with calcitonin which reverses the effects of PTH had no structural effects on joint space narrowing and although an improvement in pain and functionality, this was not deemed significant in patients with symptomatic knee OA [28]. PTH stimulates the activation of Vitamin D. Vitamin D deficiency itself has been associated with the development and worsening of knee OA [29]. Indeed supplementation of vitamin D in patients with vitamin D deficiency significantly improves knee pain and function in patients with symptomatic knee OA in comparison to placebo, although no structural changes were observed, suggesting that vitamin D may be having cellular effects that improve pain but do not yet influence bone microarchitecture [30]. Indeed it is possible that vitamin D in this study is influencing angiogenesis, as has been previously reported, to influence pain [31]. Similar results were observed in a 2 year vitamin D supplementation study in which supplementation had no effect on OA progression in patients with symptomatic OA but did however have greater effects overall on patients with lower basal levels of vitamin D [32]. This highlights the need for a larger study with a longer follow-up period, and in particular, the need to distinguish between the different subtypes of OA when in pursuit of a disease modifying treatment.

### Bone cell signalling in OA

Canonical Wnt signalling plays critical roles in a number of biological processes during development and tissue homeostasis, with pathway activation leading to increased bone formation [33]. Numerous inhibitors of this pathway have been identified including Dkk1 (Dickkopf WNT signalling pathway inhibitor 1), sclerostin, and sFRP3 (secreted Frizzled-Related Protein 3), all of which have altered expression patterns during OA development, with sFRP3 being identified as having genetic polymorphisms associated with OA development [34–36]. In humans, sclerostin mutations present as Van Buchem's disease and sclerosteosis, both of which present as a high bone mass phenotype, as does the sclerostin knockout mouse [37–39]. It is therefore unsurprising that numerous sclerostin neutralising antibodies have been developed and investigated for therapeutic intervention against osteoporosis [40–42]. However, whether such antibodies could be pursued as a target for OA treatment is somewhat a matter of contention. Studies by Roudier *et al.* have shown that sclerostin neutralising antibodies have no benefit on OA development in aged mice or in mice having undergone mechanical and surgical OA models [43]. However, it is of some concern that other studies have shown that increased Wnt signalling activation induces an OA phenotype [44]; indeed mice deficient in sclerostin have an attenuated OA pathology in response to DMM (Destabilisation of the Medial Meniscus) [45]. Similarly, overexpression of Dkk1 by intra-articular injection of Dkk1 adenoviruses significantly inhibits DMM-induced OA pathology [46]. Knowing the complexities of the Wnt signalling pathway, it is therefore unsurprising that such controversies exist and future work should perhaps focus on deciphering the precise role of Wnt activation on subchondral bone phenotype so as to avoid any off target effects through which the inhibitors of Wnt signalling may be working through.

The Wnt pathway works co-operatively with Bone Morphogenetic Proteins (BMP) which are members of the Transforming Growth Factor-β (TGF-β) superfamily unique in regulating the differentiation and function of both osteoblasts and osteoclasts to mediate bone remodelling and maintain bone homeostasis. With significant roles in AC homeostasis as well, it is therefore unsurprising that there is increasing evidence implicating both BMP and TGF-β signalling in the pathogenesis of OA. Halofuginone is a derivative of febrifugine, an ancient Chinese herbal medicine that inhibits TGF-β signalling. In mice undergoing ACLT, halofuginone accelerated subchondral bone deterioration through attenuating uncoupled subchondral bone remodelling and excessive subchondral bone angiogenesis. This was associated with the inhibition of T-helper-17-induced osteoclastogenesis and excessive TGF-β activity [47<sup>••</sup>]. Contradictory to this, inhibition of TGF-β signalling through injection of a

TGF- $\beta$  type II receptor inhibitor into the joints of ACLT mice rescued the OA phenotype [48]. It is likely that any changes in TGF- $\beta$  signalling in the subchondral bone will lead to articular cartilage degeneration as shown in TGF- $\beta$  overexpressing mice [49].

### Angiogenesis

Increased angiogenic activity leading to increased vascular invasion of the subchondral bone has been reported in the early stages of OA in both animal models and in human patients [50–53]. As such, osteochondral angiogenesis is thought to contribute to the aetiopathogenesis of OA and pharmacological agents which act to inhibit such processes are being targeted in pursuit of an OA therapy. Halofuginone, as discussed previously, acts via TGF- $\beta$  to inhibit angiogenesis through the direct inhibition of MMP2-dependent vessel formation. It is known that TGF- $\beta$  inhibition can reduce angiogenesis in subchondral bone in ACLT OA mice [48] and these results have also been observed upon administration of halofuginone [47\*\*]. Similarly, local intra-articular administration of Bevacizumab, an anti-vascular endothelial growth factor (VEGF) antibody, has beneficial results on OA development in a rabbit model of ACLT [54\*\*], whilst the injection of VEGF in to the temporomandibular joint of mice over a period of 4 weeks induces an OA phenotype with both subchondral bone and AC pathologies [55]. This therefore implicates targeting of angiogenesis in subchondral bone as an exciting new approach for future endeavours to find a disease modifying treatment for OA.

Together, this body of work highlights the potent role that the subchondral bone plays in OA aetiology. It also highlights the therapeutic potential that the subchondral bone offers when approaching targets for investigation and is in concordance with the notion that OA should be categorised into subtypes of disease.

### Targeting the synovial membrane

Synovial fibrosis is a major hallmark of OA pathogenesis, contributing to joint pain and stiffness. Various component of the synovial fibrotic cascade have been examined, including TGF- $\beta$ , Connective Tissue Growth Factor and TIMP1 (Tissue Inhibitor of MMP-1), however these were also suggested as not being attractive targets for OA therapy since these factors are known to promote anabolic responses from chondrocytes and inhibit cartilage matrix degradation; hence their inhibition might in fact accelerate AC degradation [56\*]. A better target was proposed such as PLOD2 (Procollagen-Lysine, 2-Oxoglutarate 5-Dioxygenase 2). Indeed, PLOD2 is a collagen cross-linking enzyme, which is increased during fibrosis and OA, and makes the collagen less susceptible to degradation [57,58]. Studies are yet to establish however, whether the pharmacological targeting of PLOD2 is beneficial to OA progression.

MicroRNAs are novel molecular regulators of gene expression and contribute to disease pathogenesis. These small coding RNAs represent a new class of therapeutic targets for many diseases. Some studies have used miRNA as a therapeutic agent; indeed, large quantities of miR-34a was successfully bioengineered and injected intravenously in combination with another drug against tumour growth in mice, confirming the possibility of using miRNAs as therapeutic agents [59]. Only one study to date has used intra-articular injection of miRNA [60\*\*]. Their aim was to investigate whether double-stranded miR-15a could be taken up by cells. MiR-15a was detected in the synovium, but not in the AC, suggesting miRNA may be used as therapeutic agents to treat non-cartilage tissues in OA pathogenesis such as synovial fibrosis, and might even be beneficial in avoiding negative effects on the AC. This method was subsequently used in other studies in rheumatoid arthritis studies in mice [61–63].

### Conclusions

OA is a complex multifactorial disease, which affects different tissues in the joint. To date, clinical management of OA is largely palliative with the use of opioids, Non-steroidal anti-inflammatory drugs and steroid injections with only prostheses can offer long-term aid in some individuals. Whilst numerous clinical trials have investigated various pharmacological targets, there have been varying efficacies reported and with our ever ageing population, the need for an effective disease modifying treatment is paramount. Possible avenues reside in blocking further deterioration of the AC but also on the prevention of subchondral bone thickening or treating synovial changes, with numerous targets discussed within this review. Whilst animal studies are informative, larger and more diverse clinical trials are required to investigate the true efficacy of such targets in preventing OA onset and progression.

### Conflict of interest statement

Nothing declare.

### Acknowledgements

The authors wish to acknowledge Arthritis Research UK for funding (20413 and 20859).

### References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
  - of outstanding interest
1. Goldring MB, Goldring SR: **Articular cartilage and subchondral bone in the pathogenesis of osteoarthritis.** *Ann N Y Acad Sci* 2010, **1192**:230-237.
  2. Goldring MB, Berenbaum F: **Emerging targets in osteoarthritis therapy.** *Curr Opin Pharmacol* 2015, **22**:51-63.  
Comprehensive recent review on cartilage targets for OA.
  3. Takada T, Miyaki S, Ishitobi H, Hirai Y, Nakasa T, Igarashi K, Lotz MK, Ochi M: **Bach1 deficiency reduces severity of**



- osteoarthritis through upregulation of heme oxygenase-1.** *Arthritis Res Ther* 2015, **17**:285.  
Description of HO-1 related pathway to decrease OA development in mice.
4. Cai D, Yin S, Yang J, Jiang Q, Cao W: **Histone deacetylase inhibition activates Nrf2 and protects against osteoarthritis.** *Arthritis Res Ther* 2015, **17**:269.
  5. Davidson RK, Jupp O, de Ferrars R, Kay CD, Culley KL, Norton R, Driscoll C, Vincent TL, Donell ST, Bao Y *et al.*: **Sulforaphane represses matrix-degrading proteases and protects cartilage from destruction in vitro and in vivo.** *Arthritis Rheum* 2013, **65**:3130-3140.
  6. Appleton CT, Pitelka V, Henry J, Beier F: **Global analyses of gene expression in early experimental osteoarthritis.** *Arthritis Rheum* 2007, **56**:1854-1868.
  7. Appleton CT, Usmani SE, Bernier SM, Aigner T, Beier F: **Transforming growth factor alpha suppression of articular chondrocyte phenotype and Sox9 expression in a rat model of osteoarthritis.** *Arthritis Rheum* 2007, **56**:3693-3705.
- Effect of targeting TGF $\alpha$  showing pharmacological efficacy in blocking post-traumatic OA *in vivo*.
8. Appleton CT, Usmani SE, Pest MA, Pitelka V, Mort JS, Beier F: **Reduction in disease progression by inhibition of transforming growth factor alpha-CCL2 signaling in experimental posttraumatic osteoarthritis.** *Arthritis Rheumatol* 2015, **67**:2691-2701.
  9. Yasuda T: **Cartilage destruction by matrix degradation products.** *Mod Rheumatol* 2006, **16**:197-205.
  10. Fichter M, Korner U, Schomburg J, Jennings L, Cole AA, Mollenhauer J: **Collagen degradation products modulate matrix metalloproteinase expression in cultured articular chondrocytes.** *J Orthop Res* 2006, **24**:63-70.
  11. Stanton H, Fosang AJ: **Matrix metalloproteinases are active following guanidine hydrochloride extraction of cartilage: generation of DIPEN neoepitope during dialysis.** *Matrix Biol* 2002, **21**:425-428.
  12. Chowdhury TT, Schulz RM, Rai SS, Thuemmler CB, Wuestneck N, Bader A, Homandberg GA: **Biomechanical modulation of collagen fragment-induced anabolic and catabolic activities in chondrocyte/agarose constructs.** *Arthritis Res Ther* 2010, **12**:R82.
  13. Lees S, Golub SB, Last K, Zeng W, Jackson DC, Sutton P, Fosang AJ: **Bioactivity in an aggrecan 32-mer fragment is mediated via toll-like receptor 2.** *Arthritis Rheumatol* 2015, **67**:1240-1249.
  14. Hwang HS, Park SJ, Cheon EJ, Lee MH, Kim HA: **Fibronectin fragment-induced expression of matrix metalloproteinases is mediated by MyD88-dependent TLR-2 signaling pathway in human chondrocytes.** *Arthritis Res Ther* 2015, **17**:320.
  15. Mansell JP, Collins C, Bailey AJ: **Bone, not cartilage, should be the major focus in osteoarthritis.** *Nat Clin Pract Rheumatol* 2007, **3**:306-307.
  16. Karsdal MA, Leeming DJ, Dam EB, Henriksen K, Alexandersen P, Pastoureau P, Altman RD, Christiansen C: **Should subchondral bone turnover be targeted when treating osteoarthritis?** *Osteoarthritis Cartil* 2008, **16**:638-646.
  17. Russell RG: **Bisphosphonates: from bench to bedside.** *Ann N Y Acad Sci* 2006, **1068**:367-401.
  18. Laslett LL, Kingsbury SR, Hensor EM, Bowes MA, Conaghan PG: **Effect of bisphosphonate use in patients with symptomatic and radiographic knee osteoarthritis: data from the Osteoarthritis Initiative.** *Ann Rheum Dis* 2014, **73**:824-830.
- Examines the use of bisphosphonates on the symptoms and structural progression of OA.
19. Lv Y, Xia JY, Chen JY, Zhao H, Yan HC, Yang HS, Li Q, Fan YX, Guo KJ, Chen XY: **Effects of pamidronate disodium on the loss of osteoarthritic subchondral bone and the expression of cartilaginous and subchondral osteoprotegerin and RANKL in rabbits.** *BMC Musculoskelet Disord* 2014, **15**:370.
  20. Siebelt M, Waarsing JH, Groen HC, Muller C, Koelewijn SJ, de Blois E, Verhaar JA, de Jong M, Weinans H: **Inhibited osteoclastic bone resorption through alendronate treatment in rats reduces severe osteoarthritis progression.** *Bone* 2014, **66**:163-170.
  21. Rosa RG, Collavino K, Lakhani A, Delve E, Weber JF, Rosenthal AK, Waldman SD: **Clodronate exerts an anabolic effect on articular chondrocytes mediated through the purinergic receptor pathway.** *Osteoarthritis Cartil* 2014, **22**:1327-1336.
  22. Lampropoulou-Adamidou K, Dontas I, Stathopoulos IP, Khaldi L, Lelovas P, Vlamis J, Triantafyllopoulos IK, Papaioannou NA: **Chondroprotective effect of high-dose zoledronic acid: an experimental study in a rabbit model of osteoarthritis.** *J Orthop Res* 2014, **32**:1646-1651.
  23. Nishii T, Tamura S, Shiomi T, Yoshikawa H, Sugano N: **Alendronate treatment for hip osteoarthritis: prospective randomized 2-year trial.** *Clin Rheumatol* 2013, **32**:1759-1766.
  24. Rossini M, Adami S, Fracassi E, Viapiana O, Orsolini G, Povino MR, Idolazzi L, Gatti D: **Effects of intra-articular clodronate in the treatment of knee osteoarthritis: results of a double-blind, randomized placebo-controlled trial.** *Rheumatol Int* 2015, **35**:255-263.
  25. Jaroma AV, Soininvaara TA, Kroger H: **Effect of one-year post-operative alendronate treatment on periprosthetic bone after total knee arthroplasty. A seven-year randomised controlled trial of 26 patients.** *Bone Joint J* 2015, **97-B**:337-345.
- Suggests that bisphosphonate treatment after total knee replacement may be beneficial.
26. Yan JY, Tian FM, Wang WY, Cheng Y, Song HP, Zhang YZ, Zhang L: **Parathyroid hormone (1-34) prevents cartilage degradation and preserves subchondral bone micro-architecture in guinea pigs with spontaneous osteoarthritis.** *Osteoarthritis Cartil* 2014, **22**:1869-1877.
  27. Orth P, Cucchiari M, Zurakowski D, Menger MD, Kohn DM, Madry H: **Parathyroid hormone [1-34] improves articular cartilage surface architecture and integration and subchondral bone reconstitution in osteochondral defects in vivo.** *Osteoarthritis Cartil* 2013, **21**:614-624.
- Determines that PTH treatment stimulates both articular cartilage and subchondral bone repair in an *in vivo* osteochondral defect model.
28. Karsdal MA, Byrjalsen I, Alexandersen P, Bihlet A, Andersen JR, Riis BJ, Bay-Jensen AC, Christiansen C, investigators CC: **Treatment of symptomatic knee osteoarthritis with oral salmon calcitonin: results from two phase 3 trials.** *Osteoarthritis Cartil* 2015, **23**:532-543.
  29. Heidari B, Heidari P, Hajian-Tilaki K: **Association between serum vitamin D deficiency and knee osteoarthritis.** *Int Orthop* 2011, **35**:1627-1631.
  30. Sanghi D, Mishra A, Sharma AC, Singh A, Natsu SM, Agarwal S, Srivastava RN: **Does vitamin D improve osteoarthritis of the knee: a randomized controlled pilot trial.** *Clin Orthop Relat Res* 2013, **471**:3556-3562.
  31. Grundmann M, Haidar M, Placzko S, Niendorf R, Darashchonak N, Hubel CA, von Versen-Hoyneck F: **Vitamin D improves the angiogenic properties of endothelial progenitor cells.** *Am J Physiol Cell Physiol* 2012, **303**:C954-C962.
  32. McAlindon T, LaValley M, Schneider E, Nuite M, Lee JY, Price LL, Lo G, Dawson-Hughes B: **Effect of vitamin D supplementation on progression of knee pain and cartilage volume loss in patients with symptomatic osteoarthritis: a randomized controlled trial.** *JAMA* 2013, **309**:155-162.
  33. Staines KA, Macrae VE, Farquharson C: **Cartilage development and degeneration: a Wnt Wnt situation.** *Cell Biochem Funct* 2012.
  34. Chan BY, Fuller ES, Russell AK, Smith SM, Smith MM, Jackson MT, Cake MA, Read RA, Bateman JF, Sambrook PN *et al.*: **Increased chondrocyte sclerostin may protect against cartilage degradation in osteoarthritis.** *Osteoarthritis Cartil* 2011, **19**:874-885.
  35. Loughlin J, Dowling B, Chapman K, Marcelline L, Mustafa Z, Southam L, Ferreira A, Ciesielski C, Carson DA, Corr M:

- Functional variants within the secreted frizzled-related protein 3 gene are associated with hip osteoarthritis in females.** *Proc Natl Acad Sci U S A* 2004, **101**:9757-9762.
36. Diarra D, Stolina M, Polzer K, Zwerina J, Ominsky MS, Dwyer D, Korb A, Smolen J, Hoffmann M, Scheinecker C *et al.*: **Dickkopf-1 is a master regulator of joint remodeling.** *Nat Med* 2007, **13**:156-163.
  37. Balemans W, Ebeling M, Patel N, Van Hul E, Olson P, Dioszegi M, Lacza C, Wuyts W, Van Den Ende J, Willems P *et al.*: **Increased bone density in sclerosteosis is due to the deficiency of a novel secreted protein (SOST).** *Hum Mol Genet* 2001, **10**:537-543.
  38. van Bezooijen RL, Bronckers AL, Gortzak RA, Hogendoorn PC, van der Wee-Pals L, Balemans W, Oostenbroek HJ, Van Hul W, Hamersma H, Dikkers FG *et al.*: **Sclerostin in mineralized matrices and van Buchem disease.** *J Dent Res* 2009, **88**:569-574.
  39. Li X, Ominsky MS, Niu QT, Sun N, Daugherty B, D'Agostin D, Kurahara C, Gao Y, Cao J, Gong J *et al.*: **Targeted deletion of the sclerostin gene in mice results in increased bone formation and bone strength.** *J Bone Miner Res* 2008, **23**:860-869.
  40. Ominsky MS, Li C, Li X, Tan HL, Lee E, Barrero M, Asuncion FJ, Dwyer D, Han CY, Vlasseros F *et al.*: **Inhibition of sclerostin by monoclonal antibody enhances bone healing and improves bone density and strength of nonfractured bones.** *J Bone Miner Res* 2011, **26**:1012-1021.
  41. Li X, Ominsky MS, Warmington KS, Morony S, Gong J, Cao J, Gao Y, Shalhoub V, Tipton B, Haldankar R *et al.*: **Sclerostin antibody treatment increases bone formation, bone mass, and bone strength in a rat model of postmenopausal osteoporosis.** *J Bone Miner Res* 2009, **24**:578-588.
  42. Li X, Warmington KS, Niu QT, Asuncion FJ, Barrero M, Grisanti M, Dwyer D, Stouch B, Thway TM, Stolina M *et al.*: **Inhibition of sclerostin by monoclonal antibody increases bone formation, bone mass, and bone strength in aged male rats.** *J Bone Miner Res* 2010, **25**:2647-2656.
  43. Roudier M, Li X, Niu QT, Pacheco E, Pretorius JK, Graham K, Yoon BR, Gong J, Warmington K, Ke HZ *et al.*: **Sclerostin is expressed in articular cartilage but loss or inhibition does not affect cartilage remodeling during aging or following mechanical injury.** *Arthritis Rheum* 2013, **65**:721-731.
  44. Zhu M, Tang DZ, Wu QQ, Hao SY, Chen M, Xie C, Rosier RN, O'Keefe RJ, Zuscik M, Chen D: **Activation of beta-catenin signaling in articular chondrocytes leads to osteoarthritis-like phenotype in adult beta-catenin conditional activation mice.** *J Bone Miner Res* 2009, **24**:12-21.
  45. Bouaziz W, Funck-Brentano T, Lin H, Marty C, Ea HK, Hay E, Cohen-Solal M: **Loss of sclerostin promotes osteoarthritis in mice via beta-catenin-dependent and -independent Wnt pathways.** *Arthritis Res Ther* 2015, **17**:24.
  46. Oh H, Chun CH, Chun JS: **Dkk-1 expression in chondrocytes inhibits experimental osteoarthritic cartilage destruction in mice.** *Arthritis Rheum* 2012, **64**:2568-2578.
  47. Cui Z, Crane J, Xie H, Jin X, Zhen G, Li C, Xie L, Wang L, Bian Q, Qiu T *et al.*: **Halofuginone attenuates osteoarthritis by inhibition of TGF-beta activity and H-type vessel formation in subchondral bone.** *Ann Rheum Dis* 2015.
- Suggests that inhibition of TGF- $\beta$  signalling, through administration of halofuginone, attenuates subchondral bone, and articular cartilage pathologies in an ACLT-rat model, as well as aberrant angiogenesis.
48. Zhen G, Wen C, Jia X, Li Y, Crane JL, Mears SC, Askin FB, Frassica FJ, Chang W, Yao J *et al.*: **Inhibition of TGF-beta signaling in mesenchymal stem cells of subchondral bone attenuates osteoarthritis.** *Nat Med* 2013, **19**:704-712.
  49. Jiao K, Zhang M, Niu L, Yu S, Zhen G, Xian L, Yu B, Yang K, Liu P, Cao X *et al.*: **Overexpressed TGF-beta in subchondral bone leads to mandibular condyle degradation.** *J Dent Res* 2014, **93**:140-147.
  50. Ashraf S, Mapp PI, Walsh DA: **Contributions of angiogenesis to inflammation, joint damage, and pain in a rat model of osteoarthritis.** *Arthritis Rheum* 2011, **63**:2700-2710.
  51. Saito M, Sasho T, Yamaguchi S, Ikegawa N, Akagi R, Muramatsu Y, Mukoyama S, Ochiai N, Nakamura J, Nakagawa K *et al.*: **Angiogenic activity of subchondral bone during the progression of osteoarthritis in a rabbit anterior cruciate ligament transection model.** *Osteoarthr Cartil* 2012, **20**:1574-1582.
  52. Suri S, Gill SE, Massena de Camin S, Wilson D, McWilliams DF, Walsh DA: **Neurovascular invasion at the osteochondral junction and in osteophytes in osteoarthritis.** *Ann Rheum Dis* 2007, **66**:1423-1428.
  53. Walsh DA, McWilliams DF, Turley MJ, Dixon MR, Franses RE, Mapp PI, Wilson D: **Angiogenesis and nerve growth factor at the osteochondral junction in rheumatoid arthritis and osteoarthritis.** *Rheumatology (Oxford)* 2010, **49**:1852-1861.
  54. Nagai T, Sato M, Kobayashi M, Yokoyama M, Tani Y, Mochida J: **Bevacizumab, an anti-vascular endothelial growth factor antibody, inhibits osteoarthritis.** *Arthritis Res Ther* 2014, **16**:427.
- Identifies Bevacizumab as a potential therapy for OA through inhibition of VEGF and angiogenesis.
55. Shen P, Jiao Z, Zheng JS, Xu WF, Zhang SY, Qin A, Yang C: **Injecting vascular endothelial growth factor into the temporomandibular joint induces osteoarthritis in mice.** *Sci Rep* 2015, **5**:16244.
  56. Remst DF, Blaney Davidson EN, van der Kraan PM: **Unravelling osteoarthritis-related synovial fibrosis: a step closer to solving joint stiffness.** *Rheumatology (Oxford)* 2015, **54**:1954-1963.
- Review on targeting the synovial membrane in OA development.
57. Remst DF, Blaney Davidson EN, Vitters EL, Blom AB, Stoop R, Snabel JM, Bank RA, van den Berg WB, van der Kraan PM: **Osteoarthritis-related fibrosis is associated with both elevated pyridinoline cross-link formation and lysyl hydroxylase 2b expression.** *Osteoarthr Cartil* 2013, **21**:157-164.
  58. van der Slot AJ, van Dura EA, de Wit EC, De Groot J, Huizinga TW, Bank RA, Zuurmond AM: **Elevated formation of pyridinoline cross-links by profibrotic cytokines is associated with enhanced lysyl hydroxylase 2b levels.** *Biochim Biophys Acta* 2005, **1741**:95-102.
  59. Zhao Y, Tu MJ, Yu YF, Wang WP, Chen QX, Qiu JX, Yu AX, Yu AM: **Combination therapy with bioengineered miR-34a prodrug and doxorubicin synergistically suppresses osteosarcoma growth.** *Biochem Pharmacol* 2015, **98**:602-613.
  60. Nagata Y, Nakasa T, Mochizuki Y, Ishikawa M, Miyaki S, Shibuya H, Yamasaki K, Adachi N, Asahara H, Ochi M: **Induction of apoptosis in the synovium of mice with autoantibody-mediated arthritis by the intraarticular injection of double-stranded MicroRNA-15a.** *Arthritis Rheum* 2009, **60**:2677-2683.
- One of the first studies using intra-articular injection of miRNA as a therapeutic agent targeting the synovium (and not the cartilage).
61. Nakasa T, Shibuya H, Nagata Y, Niimoto T, Ochi M: **The inhibitory effect of microRNA-146a expression on bone destruction in collagen-induced arthritis.** *Arthritis Rheum* 2011, **63**:1582-1590.
  62. Peng JS, Chen SY, Wu CL, Chong HE, Ding YC, Shiau AL, Wang CR: **Amelioration of experimental autoimmune arthritis through targeting synovial fibroblasts by the intra-articular delivery of microRNA-140-3p and -5p.** *Arthritis Rheumatol* 2015.
  63. Shibuya H, Nakasa T, Adachi N, Nagata Y, Ishikawa M, Deie M, Suzuki O, Ochi M: **Overexpression of microRNA-223 in rheumatoid arthritis synovium controls osteoclast differentiation.** *Mod Rheumatol* 2013, **23**:674-685.